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FINNEGAN HENDERSON FARABOW GARRETT &
DUNNER LLP
1300 I STREET NW
WASHINGTON DC 20005-3315

EXAMINER	
NGUYEN,Q	
ART UNIT	**PAPER NUMBER**
1632	11

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Application No.	Applicant(s)
09/359,672	BLACKBURN ET AL
Examiner	**Art Unit**
Quang Nguyen	1632

Office Action Summary

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 October 2000*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-27 and 29-50* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-27 and 29-50* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

15)☒ Notice of References Cited (PTO-892)
16)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17)☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 7
18)☐ Interview Summary (PTO-413) Paper No(s) _____
19)☐ Notice of Informal Patent Application (PTO-152)
20)☐ Other

DETAILED ACTION

Applicant's amendment filed 05 October 2000 in Paper No. 9 is acknowledged. Upon careful consideration, the examiner has decided to rejoin the invention of Group II to the elected invention of Group I. Claims 1-27 and 29-50 are pending in the present application.

Claim Objections

Claims 1, 6, 17, 33, 37 and 47 are objected to because of the following informalities: the term "dependant" is misspelled. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-27 and 29-50 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for **in vitro** methods of expressing a DNA in a cell, assaying for the effect of presence in a cell of a protein or polypeptide of DNA expression and investigating the properties of a DNA sequence, a vector for cell transfection and isolated cells transfected with the same as claimed with the following limitations, the viral replication factor is limited to polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors or SV40 large T antigen, the natural target

for polyoma large T antigen is *Ori*, and mouse ES, EC and EG cells, does not reasonably provide enablement for other embodiments of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

As written, the instant claims encompass a method of expressing a DNA in a cell, an assay for the effect of presence in a cell of a protein or polypeptide or other product of DNA expression, a method of investigating the properties of a DNA sequence by expressing a composite DNA in a cell, a vector for transfection and cells transfected with the same, for both ***in vitro*** and ***in vivo*** situations. The instant specification is not enabled for such a broadly claimed invention, because the specification fails to provide any guidance or direction regarding to claimed methods and genetically modified cells ***in vivo***. The specification provides teachings exclusively on the construction of vectors and their applications in tissue cell cultures. The nature of the claims encompassing the *in vivo* scope would fall within the realm of gene therapy which at the effective filing date of the present application was immature and highly unpredictable. The specification

fails to provide guidance or direction for a skilled artisan how to overcome obstacles known in the gene therapy art. Issues such as vector targeting, adverse host immune responses directed against administered recombinant vectors, the fate and *in vivo* expression level of the transgene provided by the delivered vectors, routes of delivery, and others have not been addressed by the instant specification. With the lack of such a guidance, it would have required undue experimentation without a predictable expectation of success for one skilled in the art to make and use the broadly claimed invention.

Regarding to the breadth of claims encompassing functional variants, analogues and derivatives of the viral replication factor selected from polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors and SV40 large T antigen, the specification does not provide any guidance or direction on which variations or modifications are made on the aforementioned viral replication factors, such that these variants and analogues and derivatives are still functionally active. Nor does the prior art provide such guidance, therefore it is incumbent upon the specification to do so. Without such guidance it would again require undue experimentation for one skilled in the art without a predictable expectation of success to make and use the broadly claimed invention. Similarly, the specification fails to provide any specific teachings regarding to functional variants of *Ori* adapted to bind to polyoma large T antigen.

With respect to claims encompassing ES, EC and EG cells derived from any and all species, apart from the specific disclosure of mouse embryonic stem cells utilized in the present claimed invention the specification fails to provide guidance or direction for

a skilled artisan how to obtain and manipulate ES, EC and EG cells derived from sources other than the mouse. It should be noted that transgenesis art utilizing the ES cell technology is limited to the mouse system because only "putative" ES cells exist for other species (Moreadith et al., J. Mol. Med. 75:208-216, 1997, page 214, Summary). In addition, Seamark (Reproduction, Fertility and Development 6:653-657, 1994) reported that totipotency for ES cell technology in many livestock species has not been demonstrated (page 6, abstract). Additionally, Mullins et al. (J. Clin. Invest. 98:S37-S40, 1996) reported that "although to date chimeric animals have been generated from several species including the pig, in no species other than the mouse has germline transmission of an ES cell been successfully demonstrated" (page S38, column 1, first paragraph). Therefore, with the lack of guidance provided by the specification, it would have required undue experimentation for a skilled artisan to make and use the broadly claimed invention.

Accordingly, due to the lack of guidance and direction provided by the instant specification, the unpredictable nature of the gene therapy art, and the breadth of the claims, it would have required undue experimentation without a predictable expectation of success for one skilled in the art to make and use the broadly claimed invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 11, 20, 23 and 37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Regarding claims 11 and 23 the phrase "such as" renders the claims indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

The term "substantially free" in claim 20 is a relative term which renders the claim indefinite. The term "substantially free" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Which of the sequences of 25, 50, 75, 100 or 200 nucleotides coding for the replication factor in the vector would be acceptable to meet the limitation of the claim? The clarity of the claim must be established, so that the metes and bounds of the claim can be determined clearly.

In claim 37, the term "capable of" is unclear and it renders the claim to be indefinite. Does the polypeptide direct or not direct the transportation of the cell active protein to the cell surface? It is suggested that the term to be deleted to overcome this rejection.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-5, 8, 12, 13, 17-21, 24, 25, 27, 29, 30 and 33-36 are rejected under 35 U.S.C. 102(b) as being anticipated by Carstens et al. (Gene 164:195-202, 1995).

The claims are directed to a method of expressing a DNA in a cell, a vector for transfection of a cell, cells transfected with the same and an assay for the effect of the presence in a cell of a protein or a polypeptide or other product of DNA expression, with specific limitations recited in the claims.

Carstens et al. disclosed a system that allows functional cloning of regulatory genes by the expression of libraries of cDNA inserts either in the sense or antisense direction using Epstein-Barr virus-based expression vectors. The disclosed system is designed to identify genes that are part of or act upon the anchorage signal transduction pathway. The system is comprised of two components (a) the library expression vectors, CMV-EL and C1E-EL, containing EB*ori*P for replication in EPNA-1 expressing cells; and (b) the EPNA-1-producing cell line BB-5, a derivative of the immortalized, non-tumorigenic and anchorage dependent human fibroblast cell line, MSU1.1. BB-5 cells supported the episomal replication of CMV-EL and C1E-EL and allowed the recovery of the vector from Hirt lysates of transfected BB-5 cells (See abstract). The vectors do not contain the EBNA-1 gene, but they comprise the sequence of hygromycin B (Hy^R) marker driven by the HSV-TK minimal promoter (See Fig. 1 and page 198, lines 4-6). Transcription of a cDNA insert in the multiple cloning site (MCS) is driven either by the cytomegalovirus (CMV) immediate early promoter/enhancer (CMV-

EL) or by the CMV immediate early promoter/enhancer containing an additional bp of first exon sequences of the CMV immediate early gene (C1E-EL). It should be noted that the disclosed vectors are adapted to contain different cDNA inserts. Carstens et al. further stated that C1E-EL was specifically designed to allow a high level transcription of mRNA from cDNA libraries inserted in the antisense orientation (page 198, column 1, lines 16-18 of the first full paragraph). Both vector constructs have been tested by inserting a luciferase cDNA into the MCS (page 198, column 1, lines 18-20 of the first full paragraph). Additionally, Carstens et al. taught the clone BB-5 was derived from the transfection of human fibroblast MSU1.1 cells with an EBNA-1 expressing vector that is presumed to contain a cryptic promoter that regulates EBNA-1 expression (page 199, column 1, lines 8-16 and Fig. 2). Carstens et al. also taught an assay to screen for transfected MSU1.1 cell colonies expressing functional EBNA-1 by measuring transcriptional transactivation of p985 which is an HSV-TK-luciferase reporter construct with the family repeats of Ebo*ri*P inserted 5' to the HSV-TK minimal promoter. In the presence of EBNA-1, the family repeats of Ebo*ri*P acts as a transcriptional enhancer. Transactivation of p985 is an indicative of functional EBNA-1 since EBNA-1 mutants incapable of supporting replication of Ebo*ri*P-containing plasmids and also do not transactivate transcription for luciferase expression (page 198, column 2, last paragraph). Upon transfecting BB-5 cells with CMV *ras*^{mut} –EL, and after 14 days of selection for Hy^R colonies, the transfected cells displayed an anchorage independent growth phenotype as colonies formed in soft agar with diameters greater than 70 μm after 21 days were observed (page 199, column 2, last sentence continues to page 200,

column 1, lines 1-8; Fig. 3). The teachings of Carstens et al. meet all the recited elements in the claims. Therefore, Carstens et al. clearly anticipates the claimed invention.

Claims 1-5, 8, 13, 17-21, 25, 27, 29 and 30 are rejected under 35 U.S.C. 102(b) as being anticipatee by Kobayashi et al. (Antisense Research and development 5:141-148, 1995).

Kobayashi et al. disclosed a transient expression assay for selection of effective antisense RNAs using episomal replication of plasmids in COS-7 cells, an African green monkey kidney-derived cell line expressing SV40 large T antigen. Plasmids expressing antisense RNAs for the retinoblastoma gene (Rb-1) mRNA and harboring SV40 *ori* were constructed and introduced into COS-7 cells to examine their inhibitory effect on the accumulation of endogenous Rb protein (See abstract). More specifically, the DNA fragments were inserted in the antisense orientation into the *apaI/XbaI* sites of a pRc/CMV mammalian expression vector containing CMV enhance-promoter unit, an SV40 ori and a bacterial neomycin resistance gene (page 142, column 1, last paragraph). Therefore, Kobayashi et al. clearly anticipates the claimed invention.

Claims 1-5, 8-12, 17-24, 27, 29-32 are rejected under 35 U.S.C. 102(b) as being anticipated by Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7).

Gassmann et al. disclosed mouse ES clone 1.19 cells that contain episomal pMGD20neo DNA and express the polyoma large T antigen. These clonal cells further support the replication, episomal maintenance and expression of hygromycin B under the control of the phosphoglycerate kinase promoter of a second plasmid PGKhphΔLT20. The second plasmid also contains the polyoma *ori*, and it does not express the replication factor polyoma large T antigen due to a 1249-bp deletion in the coding region of the large T gene (page 1295, column 1, see entire first paragraph). For further details on the disclosed pMGD20neo and PGKhphΔLT20 vector plasmids, please refer to Fig. 1. The teachings of Gassmann et al. meet all the recited elements in the claims, and therefore the reference clearly anticipates the claimed invention.

Claims 37-39, 42, 44, 49 and 50 are ejected under 35 U.S.C. 102(b) as being anticipated by Tashiro et al. (Science 261:600-603, 1993, PTO-1449 in paper no. 7).

The claims are drawn to a method of investigating the properties of a DNA sequence comprising expressing in a cell, preferably mammalian or avian cells, a composite DNA; and the same for screening a library of DNAs to identify DNA sequences coding for signal polypeptide sequences that transport protein to the cell surface, and the method optionally comprises determining whether the cell active protein is transported to the cell surface and remains there or is secreted by the cell; the same method wherein the cell active protein is a cell surface receptor. Claims 49 and 50 are drawn to the same method wherein a DNA coding for a cell surface or secreted protein or a cell surface or secreted protein are identified.

Tashiro et al. taught a method for trapping signal sequences as a strategy for cloning cDNA for growth factors and type I integral membrane proteins, taking advantage of the specific NH_2-terminal signal sequences that most precursors for secreted factors and transmembrane molecules carry and that are within 400 base pairs of the 5' termini of the mRNA (page 600, column 2, first full paragraph). Tashiro taught the construction of the pcDL-SRα-Tac(3') vector that could direct the cell surface expression of Tac (α chain of the human interleukin-2 receptor) fusion proteins when inserts with signal sequences were cloned in-frame with the correct orientation. The fusion protein expressed on plasma membranes was detected by antibodies to Tac antigen expressed on the surface of transfected COS-7 cells (See Fig. 1). The pcDL-SRα-Tac(3') vector has cloning sites between the SRα promoter and the coding sequence without a signal sequence of Tac cDNA (page 600, column 3, lines 3-10). Using this strategy, Tashiro constructed an expression cDNA library containing 5' portion-enriched cDNA of a mouse bone marrow stromal cell line ST-2, and they isolated two cDNAs that encoded putative cytokine molecules, SDF-1α and SDF-1β (See abstract). Therefore, the proteins SDF-1α and SDF-1β have been identified. The teachings of Tashiro et al. meet all the elements recited in the claims, and thus the reference clearly anticipates the instant claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 14-16 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carstens et al. (Gene 164:195-202, 1995) in view of Desnick et al. (U.S. Patent No. 5,580757).

The claims are drawn to a method of expressing a DNA in a cell in claim 1, wherein the promoter is inducible (claim 14), and wherein the transcription of the DNA can be activated by a site specific recombinase (claim 15) or wherein replication of the second vector can be prevented by a site specific recombinase (claim 16).

Carstens et al. disclosed a system that allows functional cloning of regulatory genes by the expression of libraries of cDNA inserts either in the sense or antisense direction using Epstein-Barr virus-based expression vectors. The disclosed system is designed to identify genes that are part of or act upon the anchorage signal transduction pathway. The system is comprised of two components (a) the library expression

vectors, CMV-EL and C1E-EL, containing EB*ori*P for replication in EPNA-1 expressing cells; and (b) the EPNA-1-producing cell line BB-5, a derivative of the immortalized, non-tumorigenic and anchorage dependent human fibroblast cell line, MSU1.1. BB-5 cells supported the episomal replication of CMV-EL and C1E-EL and allowed the recovery of the vector from Hirt lysates of transfected BB-5 cells (See abstract). The vectors do not contain the EBNA-1 gene, but they comprise the sequence of hygromycin B (Hy^R) marker driven by the HSV-TK minimal promoter (See Fig. 1 and page 198, lines 4-6). Transcription of a cDNA insert in the multiple cloning site (MCS) is driven either by the cytomegalovirus (CMV) immediate early promoter/enhancer (CMV-EL) or by the CMV immediate early promoter/enhancer containing an additional bp of first exon sequences of the CMV immediate early gene (C1E-EL).

However, Carstens et al. did not disclose the use of a vector wherein the promoter is inducible in their expression system. At the effective filing date of the instantly claimed invention, the use of inducible promoters in vectors is within the scope of an ordinarily skilled artisan. As an example, Desnick et al. noted that a number of vectors containing constitutive or inducible promoters can be used to express α-galctosidase as a fusion protein in yeast (column 16, lines 15-16). A specific inducible promoter that is mentioned is GAL (column 16, line 33). Therefore, the claimed method and a vector containing an inducible promoter would have been obvious given the teachings of the cited prior art in the absence of results to the contrary.

Carstens et al. taught the introduction of two *lox*P sites into CMV-EL and C1E-EL, and stated that both sites can be recombined using the P1 bacteriophage Cre

recombinase to facilitate manipulation of large cDNA-containing vectors without using restriction enzyme digestions and for manipulation of the vector without knowing the sequence of the cDNA insert (page 198, column 1, second full paragraph). It would also have been obvious to an ordinarily skilled in the art that the Cre-mediated recombination of *lox*P sites in C1E-EL would result in the generation of the EBH vector containing EB*ori*P and the vector C1E-L vector containing the CMV promoter directing the expression of a DNA but without Eb*ori*P (See bottom of Fig. 1). Under such a condition, the replication of the vector C1E-L is prevented, and by definition this vector is the second vector because it contains a DNA operatively linked to a promoter, for this instance CMV. Similarly, the reverse would also be obvious wherein the replication and transcription of a DNA in the C1E-L can be activated via Cre-mediated recombination between the second vector C1E-L and the vector EBH because of the reintroduction of Eb*ori*P into the recombined vector C1E-EL. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1, 6 and 7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7).

The claims are directed to a method of expressing a DNA of claim 1, wherein the method further comprises transfecting the cell a third vector, wherein the third vector contains a DNA. or is adapted to receive a DNA, in operative combination with a promoter for expression of the DNA, and replication of the third vector is dependent

upon presence within the cell of the replication factor, and wherein the third vector expresses a selectable marker which selectable marker is different to that expressed by the second vector.

Gassmann et al. disclosed mouse ES clone 1.19 cells that contain episomal pMGD20neo DNA and express the polyoma large T antigen. These clonal cells further support the replication, episomal maintenance and expression of hygromycin B under the control of the phosphoglycerate kinase promoter of a second plasmid PGKhphΔLT20. The second plasmid also contains the polyoma *ori*, and it does not express the replication factor polyoma large T antigen due to a 1249-bp deletion in the coding region of the large T gene (page 1295, column 1, see entire first paragraph). For further details on the disclosed pMGD20neo and PGKhphΔLT20 vector plasmids, please refer to Fig. 1. Gassmann et al. did not specifically teach an expression system wherein three independent vectors with recited limitations were introduced into a cell. However, Gassmann et al. stated that "plasmids containing a polyoma *ori*, a selectable marker, and any other gene(s) of interest could be established and maintained in such cells similarly. The capability to establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells." (page 1296, column 1, last two sentences of the second full paragraph).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the method disclosed by Gassmann et al. by further transfecting another vector containing a polyoma *ori* for episomal replication and maintenance, a different selection marker for selection purpose, and a gene of interest

operably linked to a promoter for expression in ES clone 1.19 cells previously transfected with pMGD20neo and PGKhphΔLT20 vector plasmids. Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 37. 47 and 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tashiro et al. (Science 261:600-603, 1993, PTO-1449 in paper no. 7) in view of Carstens et al. (Gene 164:195-202, 1995).

The claims are directed to a method for investigating the properties of a DNA sequence comprising expressing composite DNA in a cell system with limitations recited in claims 47 and 48.

Tashiro et al. taught a method for trapping signal sequences as a strategy for cloning cDNA for growth factors and type I integral membrane proteins, taking advantage of the specific NH_2-terminal signal sequences that most precursors for secreted factors and transmembrane molecules carry and that are within 400 base pairs of the 5' termini of the mRNA (page 600. column 2, first full paragraph). Tashiro taught the construction of the pcDL-SRα-Tac(3') vector that could direct the cell surface expression of Tac (α chain of the human interleukin-2 receptor) fusion proteins when inserts with signal sequences were cloned in-frame with the correct orientation. The fusion protein expressed on plasma membranes was detected by antibodies to Tac antigen expressed on the surface of transfected COS-7 cells (See Fig. 1). The pcDL-SRα-Tac(3') vector has cloning sites between the SRα promoter and the coding sequence without a signal sequence of Tac cDNA (page 600. column 3, lines 3-10).

Tashiro et al. did not teach the expression of the composite DNA in the cell system with characteristics recited in claims 47 and 48.

However, Carstens et al. described a system comprising of two components (a) the library expression vectors, CMV-EL and C1E-EL, containing EB*ori*P for episomal replication in EPNA-1 expressing cells; and (b) the EPNA-1-producing cell line BB-5, a derivative of the immortalized, non-tumorigenic and anchorage dependent human fibroblast cell line, MSU1.1. BB-5 cells supported the episomal replication of CMV-EL and C1E-EL and allowed the recovery of the vector from Hirt lysates of transfected BB-5 cells (See abstract). The vectors do not contain the EBNA-1 gene, but they comprise the sequence of hygromycin B (Hy^R) marker driven by the HSV-TK minimal promoter (See Fig. 1 and page 198, lines 4-6). Transcription of a cDNA insert in the multiple cloning site (MCS) is driven either by the cytomegalovirus (CMV) immediate early promoter/enhancer (CMV-EL) or by the CMV immediate early promoter/enhancer containing an additional bp of first exon sequences of the CMV immediate early gene (C1E-EL). It should be noted that the disclosed vectors are adapted to contain different cDNA inserts. Carstens et al. further taught that upon transfecting BB-5 cells with CMV ras^{mut} –EL, and after 14 days of selection for Hy^R colonies. the transfected cells displayed an anchorage independent growth phenotype as colonies formed in soft agar with diameters greater than 70 μm after 21 days were observed (page 199, column 2, last sentence continues to page 200, column 1, lines 1-8; Fig. 3).

Accordingly. it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the signal sequence trap method disclosed by

Tashiro et al. by expressing the composite DNA in the vectors and cell expression system taught by Carstens et al. to arrive at the instantly claimed invention. One of ordinary skill in the art would have been motivated to carry out the above modification because instead of investigating the properties of a DNA sequence in COS-7 cells only, the EBV-based shuttle vector system taught by Carstens et al. is applicable to most mammalian cells (page 196, column 2, lines 1-2 of the second full paragraph). Additionally, other advantages of the system disclosed by Carstens et al. include, (a) easy recovery of the vector, (b) expression level of the cDNA expression cassette is unaffected by integration, (c) no cDNA expression cassettes are lost due to disruption of the vector following integration into genomic DNA, and (d) EBNA-1, unlike SV40 large T antigen is not a transforming gene in mammalian cells, and thus allows selection for transformation-related phenotypes (page 196, column 1, last paragraph continues to the top of column 2). Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claim 40 is rejected under 35 U.S.C. 103(a) as being unpatentable over Tashiro et al. (Science 261:600-603, 1993, PTO-1449 in paper no. 7) in view of Carstens et al. (Gene 164:195-202. 1995) as applied to claims 37, 47 and 48 above, and further in view of Haub et al. (Proc. Natl. Acad. Sci. 87:8022-8026, 1990).

The claim is drawn to a method of investigating the properties of a DNA sequence comprising expressing in a cell a composite DNA with recited limitations in

claim 37, and wherein the cell active protein induces a morphological or proliferative change in the cell.

The teachings of Tashiro et al. and Carstens et al. have been presented above. However, the combined teachings did not teach a method wherein the active protein induces a morphological or proliferative change in the cell. However, it would have been obvious for an ordinary skilled artisan to substitute the coding sequence without a signal sequence of Tac cDNA with the corresponding cDNA encoding for a secreted growth factor such as FGF-3, 4, 5, 6, 7 or 8. As an example, Haub et al. disclosed cDNA sequences for both murine and human FGF-5 (See GenBank accession numbers M37821-4 and M37825, Fig. 2 and page 8026, column 1, lines 4-8). One of ordinary skill in the art would have been motivated to carry out the above modification because one can visually detect the property of a DNA sequence under investigation via a morphological or proliferative change in mammalian cells transfected with the composite DNA on the basis of a secretion of a functional growth factor molecule in culture medium, for this instance FGF-5 which has a broad cell specificity. Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 37, 45 and 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tashiro et al. (Science 261:600-603, 1993. PTO-1449 in paper no. 7) in view of Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7) and Camenisch et al. (Nucleic acids research 24:3707-3713, 1996. PTO-1449 in paper no. 7).

The claims are drawn to a method of investigating the properties of a DNA sequence comprising expressing in a cell, preferably embryonic cells, ES, EC, EG cells or differentiated progeny of such cells, a composite DNA with recited limitations.

Tashiro et al. taught a method for trapping signal sequences as a strategy for cloning cDNA for growth factors and type I integral membrane proteins, taking advantage of the specific NH_2-terminal signal sequences that most precursors for secreted factors and transmembrane molecules carry and that are within 400 base pairs of the 5' termini of the mRNA (page 600, column 2, first full paragraph). Tashiro taught the construction of the pcDL-SRα-Tac(3') vector that could direct the cell surface expression of Tac (α chain of the human interleukin-2 receptor) fusion proteins when inserts with signal sequences were cloned in-frame with the correct orientation. The fusion protein expressed on plasma membranes was detected by antibodies to Tac antigen expressed on the surface of transfected COS-7 cells (See Fig. 1). The pcDL-SRα-Tac(3') vector has cloning sites between the SRα promoter and the coding sequence without a signal sequence of Tac cDNA (page 600, column 3, lines 3-10). Tashiro et al. did not teach the expression of the composite DNA in embryonic cells, ES, EC, EG cells or differentiated progeny of such cells.

However, Gassmann et al. disclosed mouse ES clone 1.19 cells that contain episomal pMGD20neo DNA and express the polyoma large T antigen. These clonal cells further support the replication, episomal maintenance and expression of hygromycin B under the control of the phosphoglycerate kinase promoter of a second plasmid PGKhphΔLT20 plasmid. The second plasmid also contains the polyoma *ori*,

and it does not express the replication factor polyoma large T antigen due to a 1249-bp deletion in the coding region of the large T gene (page 1295, column 1, see entire first paragraph). Additionally, Gassmann taught that other plasmids containing a polyoma *ori*, a selectable marker, and any other gene(s) of interest could be established and maintained in such cells similarly. The capability to establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells." (page 1296, column 1, last two sentences of the second full paragraph). Camenisch et al. further taught that similar polyoma-based vectors were maintained extrachromosomally in differentiating ES cells and embryoid bodies as well as in established mouse cell lines (See abstract, last sentence, and pages 3711, column 2, last full paragraph and continues to page to the top of column 1 on page 3712).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the signal sequence trap method disclosed by Tashiro et al. by expressing the composite DNA in the vectors and cell expression system taught by both Gassmann et al. and Camenisch et al. to arrive at the instantly claimed invention. One of ordinary skill in the art would have been motivated to carry out the above modification because instead of investigating the properties of a DNA sequence in COS-7 cells only, the system utilized polyoma-based episomal vector taught by Gassmann et al. and Camenisch et al. is applicable to mouse differentiated and undifferentiated mouse ES cells and other established mouse cell lines. Additionally the expression level for the composite DNA would be significantly higher as noted by Gassmann et al. (page 1295, column 1, lines 14-17 of the first full paragraph)

and Camenisch et al. (See abstract, lines 9-12). Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claim 41 is rejected under 35 U.S.C. 103(a) as being unpatentable over Tashiro et al. (Science 261:600-603, 1993, PTO-1449 in paper no. 7) in view of Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7) and Camenisch et al. (Nucleic acids research 24:3707-3713, 1996, PTO-1449 in paper no. 7) as applied to claims 37, 45 and 46 above, and further in view of Williams et al. (Nature 336:684-687, 1988) and Moreau et al. (Nature 336:690-692, 1988)

The claim is drawn to a method of investigating the properties of a DNA sequence comprising expressing in a cell a composite DNA with recited limitations in claim 37, and wherein the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate.

The teachings of Tashiro et al., Gassmann et al. and Camenisch et al. have been presented above. However the combined teachings did not a method wherein the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate. Williams et al. taught that in the presence of leukemia inhibitory factor (LIF), ES cells retain the stem cell phenotype of compact colonies of small cells with a large nuclear to cytoplasmic ratio *in vitro*. whereas ES cells maintained in normal culture medium without LIF differentiate into colonies containing large, flat differentiated cells over a period of 3-6 days (page 684, column 2, last

paragraph continues to top of column 1, page 685). Moreau et al. disclosed the complete cDNA sequence for LIF (Fig. 1).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to substitute the coding nucleotide sequence of Tac cDNA lacking a signal sequence with the corresponding cDNA sequence of LIF in the modified method derived from the combined teachings of Tashiro et al., Gassmann et al. and Camenisch et al. discussed above to arrive at the instantly claimed invention. One of ordinary skill in the art would have been motivated to carry out the above modification because one can visually detect the property of a DNA sequence under investigation via the differentiated states of ES cells transfected with the composite DNA on the basis of a secretion of a functional LIF molecule in normal culture medium. Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 37, 42 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tashiro et al. (Science 261:600-603, 1993. PTO-1449 in paper no. 7) in view of Yamasaki et al. (Science 241:825-827, 1988).

The claims are drawn to a method of investigating the properties of a DNA sequence comprising expressing in a cell a composite DNA with recited limitations, and wherein the cell active protein is a cell surface receptor, preferably an IL-6 receptor.

Tashiro et al. taught a method for trapping signal sequences as a strategy for cloning cDNA for growth factors and type I integral membrane proteins, taking

advantage of the specific NH_2-terminal signal sequences that most precursors for secreted factors and transmembrane molecules carry and that are within 400 base pairs of the 5' termini of the mRNA (page 600, column 2, first full paragraph). Tashiro taught the construction of the pcDL-SRα-Tac(3') vector that could direct the cell surface expression of Tac (α chain of the human interleukin-2 receptor) fusion proteins when inserts with signal sequences were cloned in-frame with the correct orientation. The fusion protein expressed on plasma membranes was detected by antibodies to Tac antigen expressed on the surface of transfected COS-7 cells (See Fig. 1). The pcDL-SRα-Tac(3') vector has cloning sites between the SRα promoter and the coding sequence without a signal sequence of Tac cDNA (page 600, column 3, lines 3-10). Tashiro et al. did not teach a method wherein the cell active protein is an IL-6 receptor. However, Yamasaki et al. already disclosed the isolation and cloning of the human IL-6 receptor (Fig. 4). Therefore at the effective filing date of the present application, it would also have been obvious given the teachings of the cited prior art in the absence of results to the contrary that an ordinary skilled artisan would be able to substitute Tac cDNA sequence lacking the signal sequence with the corresponding cDNA sequence of IL-6 receptor. Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Conclusions

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Deborah Crouch, Ph.D., may be reached at (703) 308-1126, or SPE, Karen Hauda, at (703) 305-6608.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Patsy Zimmerman, whose telephone number is (703) 305-2758.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1632.

Papers related to this application may be submitted to Group 160 by facsimile transmission. Papers should be faxed to Group 160 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15,1989). The CM1 Fax Center number is or (703) 305-3014 or (703) 308-4242.

Quang Nguyen, Ph.D.
Examiner, AU 1632

Karen M. Hauda
KAREN M. HAUDA
SUPERVISORY PATENT EXAMINER
TECHNOLOGY